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Docket No.: 000166.0073-US02
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
J. Michael Ramstack et al.

Application No.: 10/681,142

Confirmation No.: 6453

Filed: October 9, 2003

Art Unit: 1615

For: PREPARATION OF INJECTABLE
SUSPENSIONS HAVING IMPROVED
INJECTABILITY

Examiner: S. T. Tran

DECLARATION OF M. GARY I. RILEY, D.V.M, PH.D.

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

I, M. Gary I. Riley, D.V.M, Ph.D., declare that the following facts are true:

1. I am a co-inventor of the subject matter of the above-captioned application, which is assigned to Alkermes Controlled Therapeutics, Inc., a subsidiary of Alkermes, Inc. ("Alkermes"). I am currently an employee of Alkermes, and was an employee of Alkermes during the development of the subject matter of the above-captioned application.
2. Attached as Exhibit A is a copy of notebook pages that describe a human tissue injectability test that was carried out in the United States. All dates on the notebook pages have been removed from the copy attached as Exhibit A. However, the original notebook pages of Exhibit A bear dates that are prior to May 27, 1999.

3. Exhibit A describes a two step reconstitution experiment whereby microparticles were first mixed with an injection vehicle to suspend the microparticles, and then a viscosity enhancing agent was added to increase the viscosity. As described on notebook page 1 of Exhibit A, a 3% CMC (sodium carboxymethyl cellulose) injection vehicle with a viscosity of approximately 53 cp was used to suspend blank (placebo) microspheres (microparticles) having a polymeric binder. Addition of a 10% CMC vehicle was done to increase the viscosity to approximately 730 cp. A total volume of 1 ml of the microsphere suspension was then injected through a 22 gauge needle. There were no failures of any injection.
4. Attached as Exhibit B is a copy of the results of a study carried out in the United States to investigate the effects of viscosity on microsphere injectability. All dates on the pages have been removed from the copy attached as Exhibit B. However, the original pages of Exhibit B bear dates that are prior to May 27, 1999.
5. As described on pages 1 and 2 of Exhibit B, blank (placebo) microspheres (microparticles) were suspended in an aliquot of a 3% CMC injection vehicle. The microsphere suspension was then mixed in a 3 cc syringe with increasing amounts of 10% CMC injection vehicle, the viscosity of which ranged from about 53 cp to greater than 1000 cp, depending upon the ratio of the 3% and 10% injection vehicles. Injectability of the suspensions was evaluated using thawed porcine skin and a 22 gauge needle. Three replicates were run for each viscosity with each injection rated as a success (+) or failure (-).
6. Attached as Exhibit C is a copy of a draft report of a study carried out in the United States to determine the effect of particle size, injection vehicle viscosity and injection site on the injectability of risperidone microspheres in pigs. All dates on the pages have been

removed from the copy attached as Exhibit C. However, the original pages of Exhibit C bear dates that are prior to May 27, 1999.

7. Tables 2 and 3 of Exhibit C demonstrate that a “high” vehicle viscosity resulted in a low injection failure rate, and a “low” vehicle viscosity resulted in a high injection failure rate. The “high” vehicle viscosity of Tables 2 and 3 is approximately 27 cp at 20 °C. The “low” vehicle viscosity of Tables 2 and 3 is approximately 7 cp at 20 °C. The results reported in tables 2 and 3 of Exhibit C are reflected in Tables 2 and 3 of the above-captioned application.
8. Attached as Exhibit D is a copy of a Development Report on the effect of vehicle viscosity on injectability of RISPERDAL® depot (microparticles having a poly(d,l-lactide-co-glycolide) polymeric binder and the active agent risperidone). The report provides the results of a sheep study that was conducted in the United States. All dates on the pages have been removed from the copy attached as Exhibit D. However, the original pages of Exhibit D bear dates that are prior to May 27, 1999.
9. Tables 2 and 3 of Exhibit D again demonstrate that a higher vehicle viscosity resulted in fewer injection failures, and that an injection vehicle viscosity of at least about 20 cp is necessary for successful and medically acceptable injectability rates. The experiments were conducted using high suspension concentration (greater than 100 mg/ml), and a small needle gauge size (22 gauge). At viscosities of less than or equal to about 11 cp, injectability failures increase significantly. The results reported in tables 2 and 3 of Exhibit D are reflected in Tables 4 and 5 of the above-captioned application.

10. I hereby declare that all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false or misleading statements so made are punishable by fine or imprisonment, or both, under § 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the above-captioned patent application or any patent issued thereon.

Date: May 17th 2006

M. Gary I. Riley
M. Gary I. Riley

INSTRUCTIONS

1. To secure adequate Patent-Rights is the primary purpose of this book. Only properly kept records will assure this Company of such protection. When starting a project, write in precise language your purpose, and general plan or procedure. Record your work as you progress, giving sufficient details. Handwrite directly in the book. Do not make notes on loose paper to be copied later.

2. All entries should be in ink. *Do not use pencil.*

3. The Title, project number, and book number should be accurately recorded when starting work.

4. In chronological order give a complete, accurate account of what you did, and what resulted. Enter all results, both good and bad. In case of error, draw a line through the incorrect words. Then continue with the correct wording. Copious descriptions with elaborate details are preferable. Better too much, than too little. Always keep in mind the necessity of original data to prove any new discoveries.

5. Complete calculations in detail should be written in this book and becomes our proprietary property.

6. Names of Operators, and Witnesses who are present during the demonstration should be recorded. At least one witness, not claiming to be a co-discoverer should sign and date in the space provided at bottom of work sheet. New concepts, and new solutions to problems should be witnessed by your co-workers, or someone competent to

understand the language and materials being recorded. These facts should be recorded, signed and dated.

7. New ideas, plans, procedures, sketches, etc., should be recorded immediately in this book at the time they occur. These should be *disclosed to, and understood by* your co-workers, who sign and date this fact.

8. When an experiment shows results of possible patentable importance, and No Witnesses are present, the procedure should be repeated under your supervision by your co-worker as soon as possible. Data covering the experiment should be recorded in both yours, and his Notebooks, with proper signatures and dates.

9. This Notebook and all information recorded therein are the exclusive property of this Company. All contents are strictly *Confidential*. The employee *must return* this book upon completion, upon request, or upon termination of employment. The person to whom this book is assigned, must take every precaution to safeguard against loss. In case of fire, theft, or disappearance of this book, the employee will immediately notify his supervisor. A written report describing the circumstances surrounding the loss should follow.

10. In general, only one subject should be recorded on each page. Long term projects should have separate books. All projects should be so recorded that any co-worker may continue the operation in your absence or re-assignment.

11. Pages are provided for a Table of Contents. This should be completed to enable ready access to the contents in the future.

Assigned
To

Norman Kim
DARRELL NIX

Date

Notebook
No.

-51

Returned
To

Date

By

Transferred
To

Date

By

Continued From
Notebook No.

Date

Continued To
Notebook No.

Date

TABLE OF CONTENTS

PAGE No.

i. Human tissue imitability

1

Human tissue Injectability

Objective: Determine how vehicle viscosity affects
microsphere sieving following injection. (S.C.)

Blank microspheres were used, at an injection concentration
of 200 mg/ml.

Freshly prepared 3% CMC vehicle was used to suspend
the microspheres. Addition of 10% CMC vehicle was
done to increase viscosity to approximately 730 CP.

A total volume of 1 ml of microsphere suspension
was injected through a 22 Ga TW needle.

Human tissue: NE 11-035 & NE 11-041

Photographs of the Quadrant injection
sites were taken using the Kodak Image Analyzer
and using a 35mm camera duplicate photographs
were also taken.

There were no failures of any injection. Following
a series of injections the sites were cut open
to observe the behavior of the injected suspension.
In previous experiments it was observed that
post injection sieving of the vehicle from the
microspheres was occurring. This appeared to
be an immediate event, resulting in separation of
the microsphere mass from vehicle.

In order to reduce any effects that time might

Work continued to Page

SIGNATURE

David M. M.

DATE

DISCLOSED TO AND UNDERSTOOD BY

Douglas Bissonnet

DATE

WITNESS

DATE

have on the extent of mixing two suspensions were prepared nearly simultaneously to allow injections to be made one right after another.

The first injection was the high viscosity suspension followed immediately by a lower viscosity suspension.

There appears to be a slight difference in the behaviour of the two suspensions. In the lower viscosity injection site vehicle tended to separate or sieve out away from the microsphere polymer material, leaving a slightly more dense white clump. In the high viscosity injection site the microsphere suspension remained more evenly dispersed showing little evidence of separation of vehicle from the microsphere polymer.

NOTE: 0.2 ml of green food coloring was added to 10 ml of 3% CMR vehicle to allow better visualization of the contrast between vehicle and microsphere polymer material. The food coloring doesn't turn of polymer green, it remains a white color.

A section of skin and fat layer was taken from each tissue sample that was injected and placed in 10% formalin in a heat sealed bag. These sections will be stained and processed for histopathology.

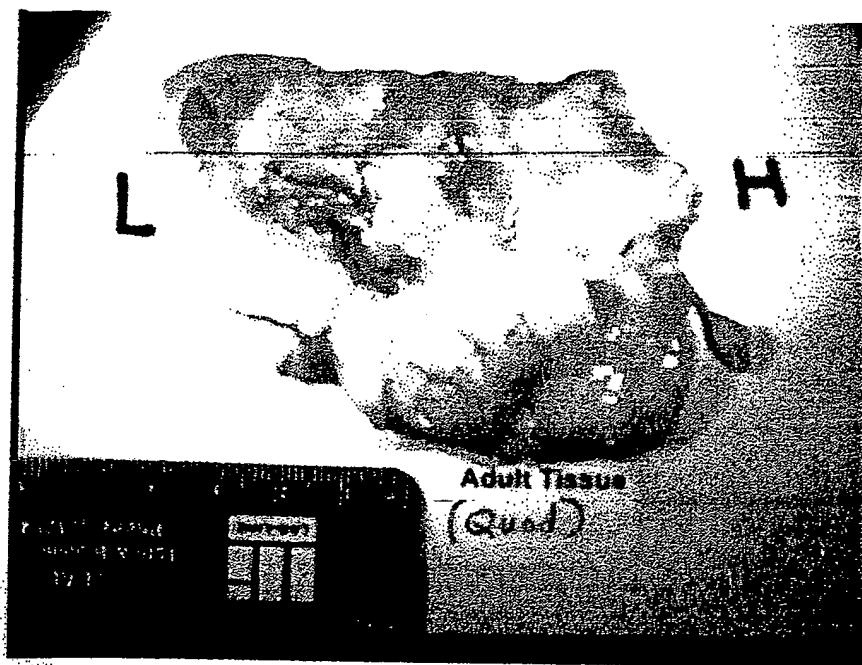
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Douglas Boonett



NE -11-035 Quadrant time following
injection of 200 μ l suspension of microsphere
blanks (in colored vehicle)

L = normal 3% CMC vehicle \sim 53 cP

H = high viscosity suspension (~~10~~ V4) \sim 730 cP

a mixture of 0.7 ml 3% CMC and 0.5 ml 10% CMC

A greater amount of separation of vehicle from
the microsphere suspension was observed in the
low vs the high viscosity vehicle suspension.

Injection sites were observed immediately following
injection. In the high viscosity site the suspension
was contained at the site until surgically opened,
at which time it began to migrate across the
entire tissue surface.

SCIENTIFIC BATTERY PRODUCTIONS, CHICAGO 60605 Made in USA

SIGNATURE

Danell Wray

Work continued to Page

DATE

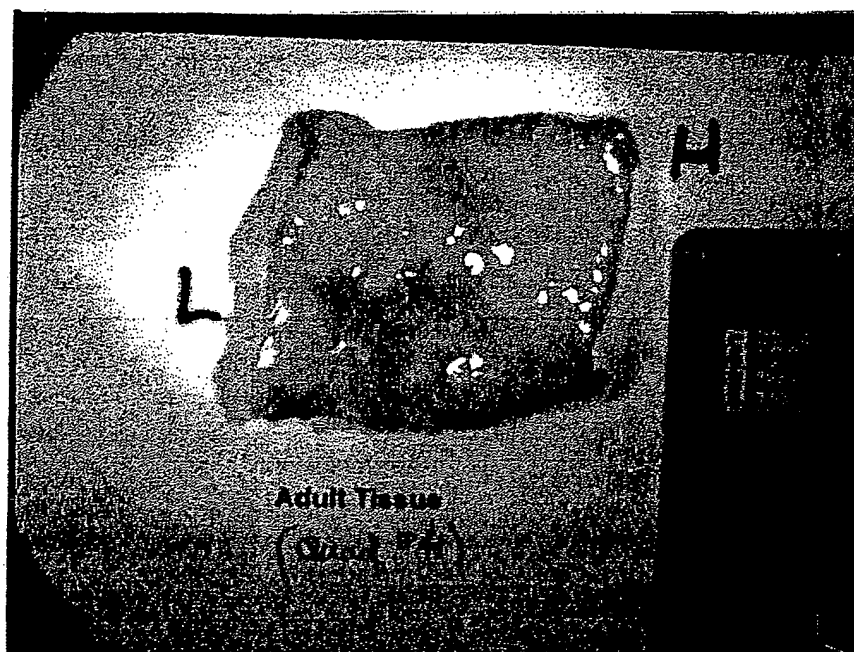
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WITNESS

DATE



NE -11-041 Quadracept tissue following injection of 200mg/ml suspension of blank microspheres (in colored vehicle).

H = high viscosity vehicle ~730 cP (V4)

L = normal 3% CMC vehicle ~53 cP

The low viscosity suspension behaved exactly the same as in patent #035 as described on previous page.

Daniel M. P.

SIGNATURE

Daniel M. P.
Douglas B. Bennett

Investigation of the effects of viscosity on microsphere injectability

Study Objective: To determine if the injection failure rate is improved by increasing the viscosity of the vehicle microsphere mixture.

Methods: Blank microspheres (300 mg) will be suspended in an aliquot of 3% CMC vehicle. The microsphere suspension will then be mixed in a 3 cc syringe with increasing amounts of 10% CMC vehicle.

| Dose (mg/mL) | 3% CMC (mL) | 10% CMC (mL) |
|--------------|-------------|--------------|
| 1. 200* | 1.2 | 0 |
| 2. 200* | 0.9 | 0.3 |
| 3. 200* | 0.7 | 0.5 |

* dose concentration assumes that the actual microspheres (300 mg) have a volume of 0.3 mL.

The viscosity will be measured using a viscometer and representative mixtures of 3% and 10% vehicle not containing microspheres.
Prior to autoclaving

Refer to table A+B over

| Vehicle Ratio (3%:10%) | % | RPM | Temp (°C) | Viscosity (cP) |
|------------------------|------|-----|-----------|----------------|
| 1. 1.2:0.0 | 10.3 | 0.6 | 25.1 | 53.1 |
| 2. 1.1:0.1 | 12.4 | 0.3 | " | 121.6 |
| 3. 1.0:0.2 | 22.9 | 0.3 | " | 234.0 |
| 4. 0.9:0.3 | 61.4 | 0.3 | " | 628.5 |
| 5. 0.7:0.5 | | | | >1000 |

* Data collected

Autoclaved

| Vehicle Ratio (3%:10%) | % | RPM | Temp (°C) | Viscosity (cP) |
|------------------------|------|-----|-----------|----------------|
| 1. 1.2:0.0 | 14.6 | 0.6 | 25.0 | 74.6 |
| 2. 1.1:0.1 | 11.8 | 0.3 | 24.9 | 120.6 |
| 3. 1.0:0.2 | 17.5 | 0.3 | 25.1 | 180.9 |
| 4. 0.9:0.3 | 20.5 | 0.3 | 25.1 | 210.5 |
| 5. 0.7:0.5 | 92.4 | 0.3 | 25.1 | 945.2 |

* Data collected

| Vehicle Ratio (3%:10%) | % | RPM | Temp (°C) | Viscosity (cP) |
|------------------------|------|-----|-----------|----------------|
| 1. 1.2:0.0 | 10.4 | 0.6 | 25.0 | 53.1 |
| 2. 1.1:0.1 | 19.3 | 0.6 | 25.3 | 99.1 |
| 3. 1.0:0.2 | 18.3 | 0.3 | 25.3 | 187.0 |
| 4. 0.9:0.3 | 24.6 | 0.3 | 25.4 | 251.4 |
| 5. 0.7:0.5 | 71.8 | 0.3 | 25.4 | 730.8 |

* Data collected

Study Protocol: Injectability

Injectability of the blank microsphere suspensions will be evaluated using thawed porcine skin. Initially three replicates will be run of each viscosity to evaluate the effects of increasing viscosity on injectability, each injection will be rated as a success or failure. Injections will be made into the upper fat layer of the porcine skin using a 22 Ga TW needle.

A

| Vehicle Ratio (3%:10%) | Injection 1 | Injection 2 | Injection 3 |
|---------------------------|-------------|-------------|-------------|
| 1. 1.2:0.0 | - | + | - |
| 2. 1.1:0.1 | - | + | + |
| 3. 1.0:0.2 | + | + | + |
| 4. 0.9:0.3 | + | + | + |
| 5. 0.7:0.5 | + | + | + |

* Data collected

B

| Vehicle Ratio (3%:10%) | Injection 1 | Injection 2 | Injection 3 |
|---------------------------|-------------|-------------|-------------|
| 1. 1.2:0.0 | + | + | - |
| 2. 1.1:0.1 | + | - | + |
| 3. 1.0:0.2 | + | + | + |
| 4. 0.9:0.3 | + | + | + |
| 5. 0.7:0.5 | + | + | + |

* Data collected

Study Protocol: Injectability

Injectability curves: A series of injectability curves will be generated for the grids below using 21, 22, and 23 Ga TW needles. Injectability will be determined using a 5x5" piece of pig flesh which has been stored at -20°C. The tissue will be thawed, and maintained at room temperature in normal saline (0.9% NaCl). Microspheres will be suspended in 0.9 mL 3% CMC vehicle, 0.9 mL of the suspension is withdrawn from vial and combined with varying amounts of a 3/10% mixture to obtain the final vehicle viscosity.

21 Ga needle

| Ratio (3:10%) | | | | | | | | | | | | | |
|-----------------|-----|--|--|-----|--|--|-----|--|--|-----|--|--|--|
| 1. 1.2:0 | | | | | | | | | | | | | |
| 2. 1.1:0.1 | | | | | | | | | | | | | |
| 3. 1.0:0.2 | | | | | | | | | | | | | |
| 4. 0.9:0.3 | | | | | | | | | | | | | |
| 5. 0.7:0.5 | | | | | | | | | | | | | |
| µs conc (mg/mL) | 175 | | | 200 | | | 250 | | | 300 | | | |

22 Ga needle

| Ratio (3:10%) | | | | | | | | | | | | | |
|-----------------|-----|---|---|-----|---|---|-----|---|---|-----|--|--|--|
| 1. 1.2:0 | - | + | + | + | - | - | - | - | - | | | | |
| 2. 1.1:0.1 | - | - | + | - | + | + | + | + | + | | | | |
| 3. 1.0:0.2 | + | + | + | + | + | - | + | - | - | | | | |
| 4. 0.9:0.3 | + | + | 0 | + | + | + | + | + | + | | | | |
| 5. 0.7:0.5 | | | | | | | | | | | | | |
| µs conc (mg/mL) | 175 | | | 200 | | | 250 | | | 300 | | | |

* Data acquired

22 Ga needle

| Ratio (3:10%) | | | | | | | | | | | | | |
|-----------------|-----|---|---|-----|---|---|-----|---|---|---|---|---|---|
| 1. 1.2:0 | | | | | | | | | | | | | |
| 2. 1.1:0.1 | | | | | | | | | | | | | |
| 3. 1.0:0.2 | | | | | | | | | | | | | |
| 4. 0.9:0.3 | | | | | | | | | | | | | |
| 5. 0.7:0.5 | X | X | X | X | X | X | X | X | X | X | X | X | X |
| µs conc (mg/mL) | 150 | | | 190 | | | 250 | | | | | | |

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Injectability of Risperidone Microspheres in Pigs: Effect of Particle Size, Injection Vehicle Viscosity and Injection Site

SUMMARY

The injectability of risperidone microspheres was evaluated in Yorkshire weanling pigs (Study AT-10-01). The objectives of the study were (1) to evaluate the utility of the weanling pig as an animal model for assessment of IM injectability of microspheres, (2) to identify critical parameters affecting injectability, and (3) to provide animal data on the effects of microsphere particle size on injectability. *more preferable*

It was found that the conditions that most closely mimic the current clinical protocol for administration (160 mg microspheres per mL vehicle; the current clinical vehicle; injection in hind quarters) did not provide a readily measurable injection failure rate (0 failures out of 10 injections). Higher failure rates were observed when a less viscous injection vehicle was employed or when microspheres were injected into a smaller muscle (i.e., the leg).

The effect of microsphere particle size on injectability was evaluated under two conditions: (1) less viscous vehicle in the hind quarter and (2) current clinical vehicle in the leg. These conditions were selected in order to provide a measurable failure rate with the current particle size distribution ($<180\text{ }\mu\text{m}$). A particle size dependence was observed under both conditions; fewer failures occurred with the $<125\text{ }\mu\text{m}$ and $<150\text{ }\mu\text{m}$ preparations compared to the $<180\text{ }\mu\text{m}$ material. The $<125\text{ }\mu\text{m}$ and $<150\text{ }\mu\text{m}$ preparations were indistinguishable with respect to the frequency of injection failure.

The data afford the following conclusions:

- The pig may provide a useful model for evaluation of risperidone microsphere injectability. However, the low failure rate observed under conditions intended to simulate current clinical practice makes it necessary to perform experiments under conditions that increase the likelihood of injection failure in order observe improvements in injectability while avoiding excessive expenditure of time and materials. Defining these conditions will require additional model development work.
- IM injectability of risperidone microspheres is dependent on injection vehicle viscosity and microsphere particle size, and to a lesser extent on the site of injection and the concentration of the microsphere suspension. Reducing the injection vehicle viscosity led to a higher rate of injection failures due to needle clogging. Lower failure rates were observed with microspheres that had been fractionated to remove particles greater than $150\text{ }\mu\text{m}$ in diameter.

MATERIALS

Test articles are listed in Table 1. Risperidone microspheres were manufactured at the 125 gram scale in the Wilmington facility. Microspheres were sized to $<125\text{ }\mu\text{m}$ and $<150\text{ }\mu\text{m}$ using USA Standard Testing Sieves Nos. 120 and 100, respectively.

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The current clinical injection vehicle (1.5% CMC, 30% sorbitol, 0.2% Tween 20) was provided by Janssen Pharmaceutica (Lot 23839). The lower viscosity vehicle (0.75% CMC, 15% sorbitol, 0.2% Tween 20) was prepared at the Blue Ash facility (Lot 96-13-103).

EQUIPMENT

19G TW x 1.5 inch hypodermic needles (B-D Precisionglide® cat. no. 305187)
3 cc hypodermic syringes (B-D cat. no. 309585)

METHODS

Animal studies were performed at Charles River Pharmservices, Inc. (Southbridge, MA). Study outlines are attached.

Injection experiments were conducted in male and female Yorkshire weanling pigs approximately 6 weeks in age (10-15 kg). Animals were anaesthetized with low doses of Telazole and Xylazine and with halothane if needed. Injection sites were shaved and cleansed with betadine swabs prior to microsphere administration.

Injections to the hind quarters were administered to the biceps femoris in the upper hind limb. Injection sites in the legs were as follows: Forelimb—superficial digital flexor muscles; hindlimb—cranial tibial muscle.

Microspheres and injection vehicle were equilibrated to ambient temperature for at least 30 minutes. Using a 3 mL syringe equipped with a 1 1/2 inch 19 gauge thin wall needle, the prescribed volume of injection vehicle was withdrawn into the syringe and injected into the vial containing microspheres. The microspheres were suspended in the vehicle by orienting the vial horizontally and rolling it between the palms of the operators hands. This was done without removing the needle/syringe from the septum. The time required to fully suspend the microspheres was approximately one minute.

The suspended microspheres were then withdrawn into the same needle/syringe and injected. Following insertion of the needle and prior to injection of the suspension, the syringe plunger was withdrawn slightly to confirm that the needle was located in the extravascular space. The time interval between aspiration of the suspension and injection was usually less than one minute.

Animals were sacrificed within approximately 24 hours following dosing. Injection regions were evaluated to pinpoint the site of microsphere deposition and to assess the distribution of microspheres in the tissue.

RESULTS

Preliminary Study: An initial range finding study was performed in order to assess the rate of injection failures under conditions approximating the current clinical practice (particle size <180 µm, current vehicle, injection in hind quarters, 160 mg microspheres per mL vehicle) and to evaluate the effects of (1) decreased injection vehicle viscosity, (2) injection in a less compliant site and (3) a twofold increase in microsphere concentration. Results are summarized in Table 2.

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No failures occurred in 10 injections performed using the clinical procedure (Table 2). Increased failure rates were observed with the lower viscosity vehicle (4 failures per 7 injections) and when microspheres were injected in the leg (1 failure per 8 injections). No failures were observed in four trials performed at the higher microsphere concentration.

Particle size effects: Table 3 summarizes injectability data for microspheres fractionated by size. Similar trends were observed when the system was stressed either by decreasing the vehicle viscosity or by injecting in the leg. In both cases, failure rates were higher with the $<180\ \mu\text{m}$ fraction. The <125 and $<150\ \mu\text{m}$ fractions were indistinguishable in terms of failure rate.

An effect of microsphere concentration was observed in the injections where $<180\ \mu\text{m}$ microspheres in the higher viscosity vehicle were administered to the leg (Table 3).

Injection site observations: Following sacrifice, injection sites in the hind quarters were examined in order to determine the location of microsphere deposition and the spatial distribution of the injected microspheres. It was observed that in most cases, microspheres were deposited intramuscularly and showed a focal and linear distribution in the tissue. In a few cases, microspheres were deposited intermuscularly and exhibited a focal distribution.

DISCUSSION

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Pig model utility: The 0% failure rate observed under current clinical conditions (Table 2) is not unexpected in view of the low failure rate observed in human patients ($<5\%$ as reported by Janssen). This suggests that the pig study procedure is not appreciably more prone to failure than administration of microspheres to human patients. Therefore, in order to detect improvements in injectability stemming from changes in formulation or methodology (e.g., reduction in maximum particle size), it is necessary either to increase the number of injections substantially or to modify the experimental conditions in order to make the system more discriminating. The former option is potentially costly in terms of time and material and in this case was not possible due to the limited quantity of microspheres available. Modifying the conditions, on the other hand, should provide useful data as long as the process leading to injection failure does not change as a result of the modification.

Based on the results in Table 2, the vehicle viscosity and the site of injection were selected as parameters to modify in order to provide conditions suitable for assessing the effects of microsphere particle size on injectability. Because the particle size effects were similar in both cases, it is unlikely that the effects are an artifact of the modified conditions.

Additional model development work will be required in order to define appropriate conditions for a given experimental objective. For example, a study aimed at optimizing injection vehicle viscosity would require modification of a parameter other than vehicle viscosity in order to ensure a measurable failure rate (e.g., site of injection).

Risperidone microsphere injectability: These studies indicate that microsphere injectability is strongly dependent on microsphere particle size and injection vehicle viscosity and somewhat dependent on the site of injection and the concentration of the microsphere suspension. The particle size effects observed are qualitatively consistent with in vitro results obtained by the Development group at Blue Ash (described in

DRAFT

Development Report dated : . On the other hand, the in vitro data do not predict the viscosity dependence observed in the animal studies.

REFERENCES

AT-10-01 Study Outlines (attached)

Notebook references:

Injectability study: Notebook -62 pp. 1-24.

Injection site characterization: Notebook! -62 pp. 58-59.

Reports:

In vitro injectability of Risperidone microspheres (Development Report dated

DRAFT

Table 1. Test articles

| Study | Description | Vial fill | Lot number |
|---------------------------|--------------|-----------|-----------------------|
| Preliminary range finding | | | |
| Phase 1, 2a, 2b | <180 μ m | 160 mg | 136-2196 ¹ |
| Phase 3 | <180 μ m | 320 mg | 136-2966 ² |
| Effect of particle size | | | |
| | <180 μ m | 320 mg | 136-2966 ³ |
| | <150 μ m | 320 mg | 136-2966 ³ |
| | <125 μ m | 320 mg | 136-2966 ³ |

¹Designated as ALK961119a in study outline

²Designated as ALK961119b in study outline

³Designated as ALK961122 in study outline

Table 2. Effect of injection vehicle, injection site and microsphere concentration on injectability¹

| Phase of study | Vehicle viscosity | Microsphere dose | Volume | Site | Failure rate |
|----------------|-------------------|------------------|--------|--------------|--------------|
| 1 | High ² | 160 mg | 1 mL | Hind quarter | 0/10 |
| 2a | High | 160 mg | 1 mL | Leg | 1/8 |
| 2b | Low ³ | 160 mg | 1 mL | Hind quarter | 4/7 |
| 3 | High | 320 mg | 1 mL | Hind quarter | 0/10 |

¹Microsphere particle size <180 μ m

²Current clinical vehicle: 1.5% CMC, 30% sorbitol, 0.2% Tween 20

³0.75% CMC, 15% sorbitol, 0.2% Tween 20

DRAFT

Table 3. Effect of particle size on injectability

| Max. particle size (µm) | Vehicle viscosity | Volume (mL) | Site | Failure rate | Avg. % delivered (failed injections) ¹ |
|-------------------------|-------------------|-------------|--------------|--------------|---|
| 180 | High ² | 2.0 | Leg | 0/5 | n/a |
| 150 | High | 2.0 | Leg | 0/5 | n/a |
| 125 | High | 2.0 | Leg | 0/5 | n/a |
| 180 | High | 1.0 | Leg | 2/4 | 0 |
| 150 | High | 1.0 | Leg | 0/4 | n/a |
| 125 | High | 1.0 | Leg | 0/4 | n/a |
| 180 | Low ³ | 2.0 | Hind quarter | 8/10 | 33 |
| 150 | Low | 2.0 | Hind quarter | 2/10 | 18 |
| 125 | Low | 2.0 | Hind quarter | 3/10 | 80 |

¹Average fraction of dose delivered prior to needle clog (failed injections only)

²Current clinical vehicle: 1.5% CMC, 30% sorbitol, 0.2% Tween 20

³0.75% CMC, 15% sorbitol, 0.2% Tween 20

DEVELOPMENT REPORT

Title: **EFFECT OF VEHICLE VISCOSITY ON INJECTABILITY OF RISPERDAL[®] DEPOT**
Prepared By: **J. Michael Ramstack
Norman N. Klm**
Date:
Product: **RISPERDAL[®] Depot**

SUMMARY

Intramuscular injectability tests were conducted in sheep in order to identify a minimum specification for vehicle viscosity to ensure successful injection of the RISPERDAL[®] Depot suspension in the clinic. Under "stressed" conditions, i.e. significantly smaller needle gauge and higher suspension concentration than expected in the clinic, vehicle viscosities ≥ 23 cp were found necessary for successful injections. Viscosities of ≤ 11 cp produced a high level of failures. Data also indicates vehicle density positively influences injectability (presumably following Stokes Law).

INTRODUCTION

The results from an earlier investigation¹ identified vehicle viscosity as a key variable affecting injectability. As part of a program to revise vehicle formulation, additional data is required in order to establish a useful minimum viscosity specification. The objective of these studies is to characterize the effect of vehicle viscosity on the *in vivo* intramuscular injectability of RISPERDAL[®] Depot and to recommend a minimum viscosity specification.

MATERIALS

RISPERDAL[®] Depot microspheres (Batch 1Kg-0908-7) were sieved through a 150 μ screen and filled in microsphere doses of 150 or 300mg into 5cc siliconized vials (Schott) and sealed with Teflon faced septum (West). The packaging materials are representative of the Phase III clinical trial product.

ProLease placebo microspheres (RG502H) were sieved through a 106 μ screen and filled in a microsphere dose of 300mg in serum/lyo flint vials (West) and sealed with gray Purcoat V-32 septum (West).

A number of vehicle formulations were prepared of various compositions. These formulations are based on mixtures of three basic starting formulations listed in Table 1. The test vehicles are listed in Tables 2 and 3. Viscosities were determined by Brookfield Model LVT viscometer fitted with UL adapter.

Animal injectability tests were conducted using 3cc syringes (B-D) and 22G TW x 1.5 inch needles (B-D) exclusively.

Table 1: Starting Vehicles

| Starting Vehicle | Lot No. | Composition |
|------------------|----------|---|
| Janssen Vehicle | 130/T-65 | 1.5% carboxymethylcellulose (CMC), 30% sorbitol, and 0.2% Tween20 |
| ProLease Diluent | 0316 | 3% CMC, 0.1% Tween 20, and 0.9% NaCl |
| Saline Vehicle | 0315 | 0.9% NaCl, 0.1% Tween 20 |

METHODS

Animal studies were conducted at Charles River Pharmservices (Southbridge, MA) using domestic sheep weighing approximately 100-150 lbs. Although earlier studies were conducted with pigs, the sheep was found a more preferred model for intramuscular injectability. Based on ultrasound and gross observation, sheep possess a greater surface area of uniform muscle thickness with a low fat content.

Animals were anesthetized with Telazole/Xylazine/Atropine intramuscularly and further supplemented with isofluorane gas (~1-2%) during the injection procedure. Prior to injection, the animal's dorsal, gluteal, and upper leg regions were shaved and cleaned with alcohol. Injection sites were visualized prior to and during dosing using ultrasound (L1 Medical).

Microspheres and vehicle were equilibrated to ambient temperature prior to dose suspension. Using a 3 cc syringe and 22 gauge thin-walled needle, vehicle was aspirated and injected into the microsphere vial. RISPERDAL Depot microspheres were suspended in 1 mL of vehicle at approximate concentrations of 130 or 230 mg/mL (~1.15 or 1.3 mL total volume). Placebo microspheres were suspended in 2 or 1 mL of vehicle at approximate concentrations of 130 or 230 mg/mL (~2.3 or 1.3 mL total volume). The vial was then agitated by hand for approximately 1 minute until microspheres were suspended. The suspension was then aspirated back into the syringe using the same needle. Care was taken to recover the maximum amount of suspension from the vial. Preparation of dose suspensions were conducted randomly by three individuals.

All doses were injected by a single individual into the animal almost immediately after preparation. The rate of injection was maintained constant at approximately 5-10 sec. Following the injection procedure, animals were euthanized by an overdose of sodium pentobarbital.

RESULTS AND DISCUSSION

The study was composed of two parts. In Part I (Phases 1 and 2) both RISPERDAL Depot and ProLease placebo microspheres were studied at two suspension concentrations of 130 and 230 mg/mL using the starting saline, Janssen or ProLease vehicles. Additional tests were conducted (Phase 3) with dilutions of the Janssen vehicle with saline. The results are reported in Table 2.

Table 2: Results of Part 1

| Phase | Treatment | Concentration (mg/mL) | Vehicle | Viscosity (cp) | Failures/Injections |
|-------|------------------|-----------------------|--------------------|----------------|---------------------|
| 1 | RISPERDAL Depot | 130 | Saline | 1 | 8/10 |
| | " | " | Janssen | 24 | 1/10 |
| | " | " | ProLease | 56 | 0/10 |
| | ProLease Placebo | " | ProLease | 56 | 1/10 |
| 2 | RISPERDAL Depot | 230 | Janssen | 24 | 0/10 |
| | " | " | ProLease | 56 | 0/10 |
| 3 | RISPERDAL Depot | 230 | 3:1 Janssen:Saline | 11 | 0/5 |
| | " | " | 1:3 Janssen:Saline | 2 | 7/10 |

In Part I, the Phase 3 vehicles were prepared by diluting Janssen vehicle with saline vehicle. This dilution represented not only changes in viscosity due to CMC dilution, but also changes in density due to sorbitol dilution. In order to study the effect of viscosity alone, additional tests (Part II) were conducted using vehicles prepared by diluting only ProLease vehicle with the saline vehicle. The results are listed in Table 3.

Table 3: Results from Part II

| Phase | Treatment | Concentration (mg/mL) | Vehicle | Viscosity (cp) | Failures/Injections |
|-------|------------------|-----------------------|--------------------------|-----------------|---------------------|
| 1 | RISPERDAL Depot | 230 | Saline | 1 | 10/10 |
| | " | " | Diluent 3 | 1 | 8/10 |
| | " | " | (1:1 Diluent 2:Saline) | | |
| | " | " | Diluent 2 | 11 | 5/10 |
| | " | " | (1:1 Diluent 1:Saline) | | |
| | " | " | Diluent 1 | 23 | 1/10 |
| 2 | " | " | (1:1 ProLease:Saline) | | |
| | " | " | ProLease | 64 | 2/10 |
| | ProLease Placebo | 130 | ProLease | 64 | 0/10 |
| | RISPERDAL Depot | 230 | Diluent 4 | 38 ¹ | 2/10 |
| | ProLease Placebo | " | (1:1 ProLease:Diluent 1) | | |
| | | | Diluent 4 | 38 ¹ | 0/5 |

¹ Estimate. Insufficient sample for measurement.

The results of the ProLease/Saline vehicle combinations in Part I and II show that vehicle viscosity has a clear effect on injectability. Based on the data, it appears viscosities at least above ~23 cp are necessary for successful injection of RISPERDAL Depot. At ~11 cp or less, injection failures increase significantly.

Comparing the 11 cp data in both Part I and II indicates that solution density may also play a role in affecting injectability outcome. The 3:1 dilution of Janssen vehicle with saline in Part I resulted in a viscosity of ~11 cp and no injection failures. Calculated sorbitol content (22.5%) of this sample, however is appreciable. Sorbitol is added to retard microsphere settling by adjusting the fluid density to better match the microspheres. Compared to this diluent in Part I, the ~11 cp sample in Part II, Dilution 2 with no sorbitol, resulted in 5/10 failures.

Injectability failure is thought to be due to microspheres separating from the mainstream of vehicle flow, accumulating and eventually causing a plug. The action of both fluid density and viscosity on injectability is consistent with Stokes Law which may, in part govern the separation process.

Suspension concentrations of 130 and eventually 230mg/mL and needle gauges of 22 TW were used in this study. In clinical practice, the maximum suspension concentration of ~100mg/mL (75mg active drug in 2mL vehicle) and needle gauge of 20 UTW is proposed for RISPERDAL Depot. Higher concentrations and smaller needle gauges were used in this study in order to "stress" the system and provide positive workable data.

The RISPERDAL Depot microspheres suspended more readily and displayed less vial adhesion and hold-up compared to the ProLease Placebo microspheres. This may be due to the vials containing RISPERDAL Depot were siliconized.

NOTEBOOK REFERENCES:

NB -16, pgs 65-66, 71

¹ Alkermes Product Development Report, Injectability of Risperidone Microspheres in Pigs: Effect of Particle Size, Injection Vehicle Viscosity, and Injection Site, AT-10-01, issued

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